



# Body Building

*Stable isotopes reveal biodiversity in the manakin diet  
that may be key to their future survival*

A QUICK GLANCE AT SOMEONE ELSE'S SHOPPING CART AT THE GROCERY STORE can convey a great deal about a person's diet. All health judgments aside, a vegetarian may have veggies, tofu, and nuts; while an omnivore might skip the tofu and add a steak. A pregnant mom may be loading up on protein, a young athlete piling on carbs, and an elderly shopper buying less food altogether. Humans and animals don't always have the luxury of choice when it comes to their diets, but when they do, it can be illuminating to see what they choose. Because you are what you eat, these choices impact the detailed composition of your body.


But it's not just about choice. Food selection for many animals is more about survival than personal preference; if a species relies too heavily on one food source, and that food becomes threatened by environmental changes or disease, the species may become threatened as well. Dietary habits may also play an important role in an ecosystem, such as by dispersing seeds or cleaning up debris. Variety in diet can translate into a better chance for survival for that species as well as those dependent upon it in the food chain.

Since scientists can't ask animals about their diets, they instead have to rely on observations of animal behavior, coupled with analysis of animal excrement, to determine what the subjects have eaten. This method is fairly reliable, but also limited because fecal samples are only representative of what was consumed recently.

Gaining popularity as an indicator of diet is the analysis of stable (nonradioactive) carbon and nitrogen isotopes assimilated into animal tissue. In all organisms, some food is consumed for energy, and some is broken down and used as building blocks for new growth. Stable isotopes help uncover clues about the latter. Scientists at Los Alamos recently applied their expertise in stable isotopes to analyze bird feathers from Ecuadorian manakins, small, fruit-loving birds who distribute seeds in their Amazon habitat—

a key ecosystem service. Using a specialized algorithm developed to incorporate uncertainty in the isotopic concentrations from

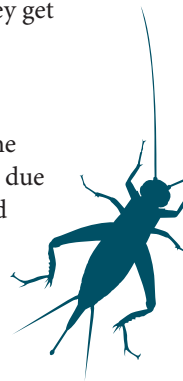




each food source, the scientists were able to thoroughly examine the diet of the birds and the destination of the atoms they get from their food.

### You are what your food ate

Hidden between the lines of the periodic table are the isotopes—different versions of the same element, each with a slightly different mass due to their nuclei having additional or missing neutrons. For decades, scientists have measured and exploited the decay rate of unstable isotopes. For example, carbon-dating of materials is done by examining the ratio of stable  $^{12}\text{C}$  (read “carbon-12”) to unstable  $^{14}\text{C}$ , which has a half-life of nearly 6000 years. Some stable isotopes—especially relatively rare isotopes of life-sustaining elements such as carbon, hydrogen, oxygen, or nitrogen—are also useful in biological research as they vary predictably by geographic region and can be used as signatures of an organism’s lifestyle.



For instance, rainwater—and therefore drinking water—in coastal areas is enriched with more of the isotope oxygen-18 than that from inland areas and higher elevations. This is because raindrops containing  $^{18}\text{O}$  tend to precipitate first, before the much more common  $^{16}\text{O}$ , and as rainstorms move inland from the coast, fewer  $^{18}\text{O}$  water molecules remain in the cloud, in comparison to  $^{16}\text{O}$  and  $^{17}\text{O}$ . (All three isotopes are stable, and the differences are miniscule but measurable.)

Plants are often characterized by their specific method of converting carbon dioxide ( $\text{CO}_2$ ) to sugar and other carbohydrates—a process called carbon fixing. So-called “ $\text{C}_4$  plants” include corn and sugar cane, and the more common  $\text{C}_3$  plants include rice and wheat. Because of a difference in the enzyme used in  $\text{C}_4$  plants, they accumulate slightly more  $^{13}\text{C}$ . As a result, people in North America who eat more corn (or corn-fed beef) have a higher  $^{13}\text{C}$  isotope profile.

“You can measure differences in isotopic composition that exist along a human hair that may indicate that a person has moved from one [geographic] region to another, based on the isotopes assimilated from their diet,” explains Dave Podlesak, a chemist who is involved with a number of stable isotopes projects at Los Alamos.

He says that for years forensic scientists have been evaluating stable isotopes in hair and fingernails, which are constantly growing and incorporating new atoms, thereby keeping a record of the available isotopes.

Forensic scientists must keep in mind that an organism’s isotope profile comes primarily from the food and water consumed but also can be impacted by its trophic level, or place in the food chain. With nitrogen, for example, organisms tend to preferentially



excrete  $^{14}\text{N}$  (the most abundant form of nitrogen) and accumulate  $^{15}\text{N}$  because the lighter isotope reacts slightly faster due to lower bond strength and is, therefore, excreted before heavier ones, which are often assimilated. When one organism eats another, the  $^{15}\text{N}$  accumulation is compounded. In the same manner that a high-level predator such as a tuna fish can have a greater concentration of mercury than other animals by accumulating it from many trophic levels of its prey, an organism cumulatively gathers isotopically heavier nitrogen from its food sources as well.



“In general, each trophic level enriches the ratio of  $^{15}\text{N}$  to  $^{14}\text{N}$  by about 3 per mille [parts per thousand],” says Jeanne Fair, a biologist who led the Los Alamos portion of the manakin research.

## Sticky situation

The Amazon rainforest is one of the most biologically diverse places on the planet. It is home to hundreds of bird species, many of which are crucial for ecosystem function, as they disperse seeds and nutrients, pollinate flowers, eat pests, and scavenge on forest debris. These ecosystem services, in turn, are critical for the health of the forest; the plants that grow as a result of the birds’ seed dispersal are food for other animals; and those animals may be food for larger predators. The rapid loss of tropical birds due to climate or environmental changes may significantly disrupt their ecosystems.

For the last ten years, the National Science Foundation (NSF) has funded a multi-institutional study of manakin birds in their habitat in the Amazon. The studies have been done at the Tiputini Biodiversity Station, in the Yasuni Biosphere Reserve in Ecuador. The Reserve is a haven of biodiversity and is home to nearly 600 bird species and more than 100,000 different species of insects, not to mention amphibians or mammals. The Reserve also happens to be located atop an estimated hundreds of millions of barrels of crude oil, making conservation of the rainforest the subject of heated debate. In fact, in 2007, Ecuadorian President Rafeal Correa offered to protect a significant sub-section of the drilling area in hopes that the global community would contribute funds to his country in lieu of the oil revenue Ecuador would be giving up (as gratitude for the green gesture). But he abandoned the idea in 2013 after only \$13.3 million had been received out of \$300 million pledged—both far less than the \$3.6 billion he had hoped for.

Because of their role in seed dispersal, manakins are very important to the welfare of the Amazon forest. The researchers in the NSF study, who are from the University of Florida, Gainesville, and the Smithsonian Migratory Bird Center in Washington, D.C., hope to find out more about them before the drilling permanently changes their ecosystem.

“If you take the manakins or other seed-dispersing species out

of the forest, the forest will change, and we have no idea how to predict those changes,” says Fair.

One aspect of their investigation has been to study the diet of six species of manakins that live closely together in the area near the Tiputini station. Among the advantages of studying manakins is that they are neither migratory nor live in a particularly unstable environment (yet), both of which can alter stable isotope assimilation. Understanding more about their diet could help the team evaluate their vulnerability in the changing Amazon.

The diet analysis took place over a period of two years, during which the team captured and examined 147 individual birds representing all of the six species. Upon capture, a small length (2–3 millimeters) of the tip of the middle tail feather was clipped for stable isotope analysis; fecal samples were routinely collected as well. The scientists also observed the birds foraging and took samples of what they were eating: plants with ripe fruit and insects such as crickets, ants, and larvae. It was previously known that frugivorous (fruit-eating) birds often complement their diet with insects to obtain protein. The investigators hoped the stable isotope analysis at Los Alamos would help quantify how much of the manakins’ diet is actually made up of insects.

## Early bird catches the...

In order to understand the manakin diet, the team analyzed both the assimilated  $^{15}\text{N}$  and  $^{13}\text{C}$  in the bird feathers and the isotope ratios present in each food source. First, the team dried and homogenized the feathers, fruits, and insect samples before they were fed into an analyzer, which burned and purified the organic matter into  $\text{CO}_2$  and  $\text{N}_2$ . Finally, a mass spectrometer ionized the gases and separated the isotopes for quantification.





“It would have been very easy to look at these absolute data [on isotopic concentration] and see that they didn’t correlate with the fecal and foraging data, but we wanted to be more thorough and decided to add the mixing model approach,” explains Jeff Heikoop, a geochemist who, along with colleague George Perkins, completed the isotopic analysis at Los Alamos. Heikoop and Perkins decided to use an isotopic mass-mixing model, which they had previously used in another project evaluating river water for evidence of nuclear processing. Los Alamos sub-contractor Paul Davis developed the algorithm.

“We used this mixing model to look at the isotope profile of rivers for the Global Security program at Los Alamos,”



-23.9  $\delta^{13}\text{C}$  ‰, 9.45  $\delta^{15}\text{N}$  ‰

### Sample $\delta^{13}\text{C}$ ‰ $\delta^{15}\text{N}$ ‰

	-32.1	1.3
	-25.0	3.7
	-27.8	4.0
	-33.0	5.8

Each component of the manakin diet was measured for its individual stable isotope profile. As is standard in all stable isotope research, the raw numbers are given context by comparison to an accepted calibration protocol: atmospheric nitrogen for nitrogen, and a specific fossil carbonate for carbon. For example, a measurement of  $^{15}\text{N}/^{14}\text{N}$  in a larvae is written  $\delta^{15}\text{N} = 5.8$  ‰ indicating the larvae has a measurement of 5.8 parts per thousand more  $^{15}\text{N}/^{14}\text{N}$  than the ratio of the same nitrogen isotopes in the atmosphere. The profiles of the food sources were then compared to the profiles of feather clippings from all the captured birds.



says Julianna Fessenden-Rahn, a chemist in the Defense Security Analysis Division, who led that project. “We tried to find signatures of nuclear processing hidden among isotopes from other sources such as fertilizer effluents.” For both the river analysis and the manakin study, the challenge was to identify the ranges of values from each source and how they could mix or change in the system being analyzed (a river or a bird).

The manakin research team corrected for the trophic level isotope effect for fruit and insects before using the data in the mixing model. Next, the algorithm incorporated the effects of isotopic uncertainty from the food sources in a way other models would not have allowed. “We were able to look at two isotope systems in each feather sample,  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$ , and find dietary mixing solutions consistent with both,” says Heikoop.

In the end, the model gave them 5.9 million possible combinations of fruit and insects that could match the isotope ratios found in the feathers. The results broadly indicated a greater percentage of assimilated  $^{15}\text{N}$  coming from insects as the food source. This supported the notion that the manakins are not completely frugivorous and that they eat a significant amount of insects to obtain more protein.

Nitrogen is a key component of amino acids, the building blocks of protein, and protein is a major component of feathers. With this in mind, the team’s data might underestimate the amount of fruit in the manakin diet because it may have been mostly used for energy, instead of for making feathers. The researchers also realized the birds must get a large proportion of nitrogen from larvae, spiders, and other soft-bodied arthropods that do not leave indigestible exoskeleton remains in their feces and are, therefore, missing in the fecal analysis. When the stable isotope data were combined with the observational and fecal data, the team was able to construct a comprehensive picture of the manakin diet and the assimilation of nutrients into their feathers.



A *Pipra filicauda* manakin in Ecuador’s Yasuni Biosphere Reserve

## So they’re not herbivores

Confirmation that manakins do indeed consume insects is a valuable data point towards understanding their fate in a changing ecosystem.

“Some of the questions we as researchers ask are, ‘What is going to drive adaptability to climate change?’ and ‘Are these species capable of rapid evolution?’” says Fair.

Since the manakins vary their diet, they may be less vulnerable to extinction if their surrounding environment is altered—which is good news, considering their important role in the forest. Other species, however, may not be as adaptable, and Fair explains that, if identified, those more fragile species could become the recipients of focused conservation efforts. While the whole goal is to preserve as much biodiversity as possible, species that provide critical ecosystem services may be especially important—not just to a particular ecosystem, but to humanity as well.

“Biodiversity is the best predictor of resilience and adaptability,” says Fair. “The fundamental truth is that biodiversity matters profoundly to human health in almost every conceivable way. The roles that species and ecosystems play in providing food, fuel, and unique medicinal compounds; the purification services for air, water, and soil; and the natural regulation of infectious disease, to name a few, are critical to our health and survival.”

—Rebecca McDonald

## Isotopes in action

Stable isotopes have been a part of research at Los Alamos for nearly five decades. In the 1960s, scientists at the Lab were the first to create and automate large-scale separation of  $^{13}\text{C}$  from  $^{12}\text{C}$ . This enabled researchers to introduce stable isotopes to a system as tracer molecules or labels in research, as opposed to measuring stable isotopes that naturally exist in an organism.

“Stable-isotope labeling is valuable for evaluating chemical and biochemical reactions and for analyzing metabolites, proteins, and other biomolecules,” says Los Alamos biochemist Cliff Unkefer. When possible, stable isotopes are useful in place of radioisotopes that may have adverse effects on the study.

Stable isotopes can also be used in medical tests. The diagnostic test for *Helicobacter pylori*, the bacterium that causes ulcers, uses urea labeled with  $^{13}\text{C}$  as a tracer. Patients swallow the tracer (dissolved in orange juice) and when the *H. pylori* break down the urea into ammonia and  $\text{CO}_2$ , the  $^{13}\text{C}$  can be detected in the patient’s breath.